

CLAIMS

1) A method for the design and/or the selection of chemokines variants having agonist or antagonist property towards a ligand of GPCR of animal cells comprising the following steps:

- 5 A) obtaining a phage displayed library expressing on their surface said chemokine variants mutated within the domain responsible for their effector function,
- B) having a culture of animal cells expressing on their membranes the GPCR,
- 10 C) Incubating the cell culture with the phage library obtained in A),
- D) harvesting the cells after removal of non specifically bound and surface receptor bound phages,
- E) Releasing the phages internalized in step C) by lysis of cells obtained in D)
- 15 F) Infecting an *E. coli* culture with the released phages obtained in E) and amplifying the clones previously internalized ,
- G) Obtaining a phage library enriched in internalizing chemokines ligands,
- 20 H) Assaying the agonist or antagonist property of the chemokine variants versus the native one.

2) The method according to claim 1 wherein the chemokine is
25 RANTES.

3) The method according to claim 1 wherein the GPCR expressed within the membrane of animal cells is CCR5.

30 4) The method according to claim 1 wherein the animal cells are human cells.

5) The method according to claim 2 wherein the phage library of RANTES variants is obtained using a method comprising the following steps:

- 5 - Obtaining a DNA sequence coding for human RANTES resulting from the amplification of cDNA prepared from activated PBMCs,
- Performing a PCR mutagenesis of the 5'portion of the DNA sequence of RANTES using a specific downstream primer and
10 a degenerate upstream primer containing recognition sites for restriction enzymes in order to insert the PCR amplification products into the phage display vector,
- Inserting the purified PCR products into a phage display vector,
- 15 - Production of the phage library by introducing the vector containing the purified PCR products into an *E. coli* culture.

6) The method according to claim 2 wherein anti-HIV activity is assayed.

7) A method for the design and/or the selection of chemokines having agonist or antagonist property towards a GPCR of animal cells comprising the following steps:

- 25 A) obtaining a phage displayed library expressing on their surface said chemokine mutated within the domain responsible for their effector function,
- B) having a culture of animal cells expressing on their membranes the GPCR,
- 30 C) Incubating the cell culture with the phage library obtained in A),

D) Eliminating the non specifically bound phages from the cells, by a process keeping the specifically bound phages on the said receptor

E) Incubating the cells obtained in D) with an *E. coli* culture and amplifying the clones being infected by the phages bound to the said receptor on animal cells,

F) Obtaining a phage library enriched in externally bound phages,

G) Assaying the agonist or antagonist property of the chemokine variants versus the native chemokine.

8) The method according to claim 7 wherein the chemokine is RANTES.

9) The method according to claim 7 wherein the GPCR expressed within the membrane of animal cells is CCR5.

10) The method according to claim 7 wherein the animal cells are human cells.

11) The method according to claim 8 wherein the phage library of RANTES variants is obtained using a method comprising the following steps:

- Obtaining a DNA sequence coding for human RANTES resulting from the amplification of cDNA prepared from activated PBMCs,
- Performing a PCR mutagenesis of the 5'portion of the DNA sequence of RANTES using a specific downstream primer and a degenerate upstream primer containing recognition sites for

restriction enzymes in order to insert the PCR amplification products into the phage display vector,

- Inserting the purified PCR products into a phage display vector,
- Production of the phage library by introducing the vector containing the purified PCR products into an E. coli culture.

12) The method according to claim 8 wherein anti-HIV activity is assayed.

13) A compound obtainable by a method according to anyone of claims 1 to 12 of the following formula: *SP#SSQ&&&-RANTES(10-68), in which

- * is L or an aromatic residue,
- # is L, M or V
- & is S, P, T or A.

14) The compound according to claim 13) having one of the following formulae :

LSPVSSQSSA (P₁)

FSPLSSQSSA (P₂)

LSPMSSQSPA

WSPLSSQSPA

WSPLSSQSSP

LSPQSSLSSS

ASSGSSQSTS

ISAGSSQSTS

RSPMSSQSSP

YSPSSSLAPA

MSPLSSQASA

ASPMSSQSSS
QSPLSSQAST
QSPLSSTASS
LSPLSSQSAA
5 GSSSSSQTPA
YSPLSSQSSP
FSSVSSQSSS,
VSTLSSPAST,
ASSFSSRAPP,
10 QSSASSSSSA,
QSPGSSWSAA,
QSPSSWSSS,
QSPLSSFTSS,
LSPQSSLSSS,
15 ASPQSSLPAA,
LSPVSSQSSA

15) The compound according to claim 13) having the formula:
FSPLSSQSSA-RANTES(10-68).

20 16) The compound according to claim 13) having the formula:
LSPVSSQSSA-RANTES (10-68).

17) A pharmaceutical composition which comprises of a
compound having the formula *SP#SSQ&&&-RANTES(10-68), in which

- 25
- * is L or an aromatic residue,
 - # is L, M ouV
 - & is S, P, T or A,
- or a pharmaceutical salt thereof, in a mixture with one or more
pharmaceutically acceptable excipient.

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18) The composition of claim 17) in which the compound have the formula: LSPVSSQSSA-RANTES(10-68).

5 19) The composition of claim 17) in which the compound have the formula: FSPLSSQSSA-RANTES(10-68).

20) A method for preventing and/or inhibiting HIV infection in humans comprising a step of treatment with a composition of claim 18).

10 21) A method for preventing and/or inhibiting HIV infection in humans comprising a step of treatment with a composition of claim 19).

15 22) A method for preventing and/or curing inflammatory or malignant diseases in humans comprising a step of treatment with a composition of claim 13 or 14.